REMARKS

Claims 1-16 are pending. No new matter has been added by way of the above amendments. For example, claims 1 and 17 have been amended to recite "purified" biologically active protein and "purified" biologically active interferon, respectively. These amendments are supported by claim 5 as well as the present specification at page 5, lines 20-21 and page 7, lines 8-9. Claims 9 and 10 have been amended in order to clearly recite whether the solutions are prefiltered or sterile filtered before or after the detergent is added. These amendments are supported by the present specification at, for instance, page 7, lines 12-15. The present specification at page 2 has been amended to reflect the nature of the trademarked products recited therein. Lastly, the present specification at page 4 has been amended to remove reference to claims 1, 13 and 15. These amendments are supported by originally filed claims 1, 13 and 15. Accordingly, no new matter has been added.

Applicants further submit that no new issues have been raised by way of the present submission. For instance, Applicants have simply amended claim 1 to recite "purified" biologically active protein and claim 17 to recite "purified" biologically active interferon. This is not a new issue that would require additional search and/or consideration on the part of the Examiner. For example, this issue was already searched and considered with

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respect to claim [illegible]. In the event that the present amendment does not place the application in condition for allowance, entry thereof is respectfully requested as placing the application in better condition for appeal.

In view of the following remarks Applicants respectfully request that the Examiner withdraw all rejections and allow the currently pending claims.

Objections to the Specification

The Examiner has noted the use of trademarks at page 2 of the specification. Applicants traverse and submit that these trademarks have been placed in capital letters. Accordingly, this objection is moot. Reconsideration and withdrawal thereof are respectfully requested.

The Examiner has also objected to the specification for the referencing of claims on page 4. Applicants note that claims 1, 13 and 15 are referenced on page 4. The present amendment replaces these references with the relevant subject matter of originally filed claims 1, 13 and 15. Accordingly, this objection is moot. Reconsideration and withdrawal thereof are respectfully requested.

Issues Under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims [illegible] and [illegible] under 35 U.S.C. §112, second paragraph asserting that it is unclear whether the

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[illegible] prefiltered, sterile filtered, [illegible] after the detergent is added. Applicants respectfully traverse this rejection.

A review of the present specification, for instance at page 7, lines 12-15, indicates that the step of prefiltering occurs after the detergent is added. The sterile filtration step occurs prior to recovery. Applicants have amended claims 9 and 10 to reflect this relationship. Accordingly, this rejection is moot. Reconsideration and withdrawal thereof are respectfully requested.

Issues Under 35 U.S.C. §103(a)

The Examiner has rejected claims 1-18 under 35 U.S.C. §103(a) as being obvious over Georgiades '683 in view of Manabe '315. Applicants respectfully traverse this rejection.

Distinctions Between the Present Invention and the Cited Art

As pointed out by the Examiner, Georgiades '683 discloses the addition of detergent as a means of virus inactivation. Georgiades additionally discloses that membrane filtration is an additional means of viral inactivation. In this regard, refer to the end of the summary, column 4, lines 1-8 of Georgiades '683. A detailed inspection of Georgiades '683 reveals that the membrane filtration referred to by Georgiades '683 is ultrafiltration with [illegible] molecular weight cut-off membranes, which are passed by interferons. The filtration of crude interferon through such

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membranes in the presence of nonionic detergent is disclosed. However, it is not demonstrated that the filtration through the ultrafiltration membranes would eliminate viruses. Virus elimination is only studied in Example 10, which demonstrates that no infectious Sendai virus was left after the detergent treatment and no additional effect was demonstrated for the membrane filtration. In fact, it is currently known that ultrafiltration with typical ultrafiltration membranes is not an effective and reliable method for virus elimination.

Efficacious virus removal by membrane filtration became possible first after development of suitable membrane materials, such as the porous hollow fiber membrane described in the Manabe '315 patent. As stated in the background of the Manabe '315 patent (col. 2, lines 17-27), the virus removal percentage normally required for a virus removal membrane is as high as 99.99 to 99.99999%. Effective virus elimination by membrane filtration therefore requires specific membrane construction that differs greatly from ultrafiltration membranes such as used in Georgiades '683. Differences in ultrafiltration and virus removal filters is further described in Appendix 1.

In addition to the difference in the type of filtration, a major difference between the membrane filtration described in Georgiades '683 and the present application is that in the Georgiades '683 crude, only partially purified interferon solution is filtered, while in the present application purified interferon

[illegible] is important, because impurities in crude protein solutions tend to block virus removal filters and it is beneficial to have the virus removal step at the end of the purification process of biologically active proteins to prevent recontamination and ensure viral safety of the finished product. This is analogous to sterile filtration that is meant to remove residual bacterials just before aseptic filling. Because pure interferons, like several other hydrophobic proteins, are sticky and tend to aggregate and adhere to surfaces, albumin is commonly added to stabilize pure protein solutions. This is described for a beta-interferon preparation in Reference 1. Albumin is also added to stabilize the interferon preparation described in Georgiades '683 (col. 6, lines 12-15: "Following the concentration on 10,000 molecular weight membrane, human serum albumin is added as a stabilizing agent for interferon alpha at a concentration of 1 mg to 10 mg per ml, preferably 5 mg").

The current application demonstrates that the filtration of albumin-containing solutions is inefficient due to rapid blocking of the membrane. An unexpected discovery of the present invention is that when non-ionic detergents are used instead of albumin to stabilize the purified protein solution, virus filtration can be performed with much better filter performance (constant flux of filtrate, no fouling of the filter) while maintaining efficient virus removal (virus elimination described in Table 1 of the

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Appeal [illegible]

In summary, the presently claimed subject matter wherein a purified biologically active protein is subjected to a non-ionic detergent and then later filtration on a virus removal filter is neither suggested nor disclosed by the cited references. As discussed above, according to the present invention, when non-ionic detergents are used instead of albumin, for the stabilization of purified protein solutions, virus filtration can be performed with superior filter performance while maintaining efficient virus removal. This was explained in Appendix 1.

Accordingly, the Examiner has failed to present a valid *prima facie* case of obviousness. Moreover, even if the Examiner has hypothetically presented a *prima facie* case of obviousness, the unexpected results of the present invention rebut any *prima facie* case of obviousness. The Examiner is therefore respectfully requested to withdraw all rejections and allow the currently pending claims.

If the Examiner has any questions or comments, please contact Craig A. McRobbie, Registration No. 42,874 at the offices of Birch, Stewart, Kolasch & Birch, LLP.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional

fees required under 37 C.F.R. § 1.16 or under § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By ______________________

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Attachment: Version with Markings to Show Changes Made
Appendix I

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph on page 2, lines 14-24 has been amended as follows:

--In order to prevent the binding of biologically active proteins, such as IFN-α, to filters, final containers and other surfaces, stabilizers are typically added to solutions containing the purified biologically active protein. In additional to the above short-term stabilizing effect, stabilizers will also prevent aggregation of the proteins and, thus, provide extended shelf-life. Albumin is the most common stabilizer used, e.g., in multicomponent IFN-α products and it is employed in many of the commercial preparations [(Alfanative®, Alferon® N, Wellfoeron®)] (ALFANATIVE®, ALFERON® N, WELLFOERON®).--

The paragraph on page 4, lines 17-19 has been amended as follows:

--In particular, the present method for preparing virus safe pharmaceutical compositions of biologically active proteins is characterized by [what is stated in the characterizing part of claim 1] a method of preparing a pharmaceutical composition of a biologically active protein comprising the steps of 1) adding to [illegible]

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the solution containing the non-ionic detergent by filtration [illegible] a virus removal filter with a pore size of 1 to 50 nm; and [illegible] recovering the filtrate.--

The paragraph on page 4, lines 21-24 has been amended as follows:

--The method for stabilizing pharmaceutical compositions of purified leukocyte α-interferon is characterized by [what is stated in the characterizing part of claim 13] an α-interferon composition, comprising a non-ionic detergent as a stabilizer in an amount exceeding the critical micellar concentration of the detergent and being essentially free from substances and agents retained on a virus-filter having a high virus retentive capacity even for small non-enveloped viruses and the virus-safe α-interferon solution is characterized by [what is state in the characterizing part of claim 15] a composition comprising an α-interferon solution containing at least two α-interferon subtypes selected from the group consisting of α1, α2, α4, α7, α8, α14, α16 and α21.--

In the Claims:

The claims have been amended as follows:

Claim 1. (Twice Amended) Method of preparing a pharmaceutical composition of a purified biologically active protein, comprising

[illegible]

[illegible] of the purified protein a non-ionic detergent;

subjecting the solution containing the non-ionic detergent to filtration on a virus removal filter with a pore size of 10 to 40 nm; and

recovering the filtrate.

Claim 9. (Twice Amended) The method according to claim 1, further comprising prefiltering [a proteineous solution] said solution obtained in step (1) with a 0.04-[illegible] µm filter[, then filtering it with a virus removal filter having a pore size of 10-40 nm,] prior to said step (2) and [finally] subjecting the filtrate obtained in step (2) to sterile filtration[, and recovering the filtrate].

Claim 10. (Twice Amended) The method according to claim 1, further comprising sterile filtering said solution obtained in step (1) [a proteineous solution and subsequently subjecting the filtrate of the sterile filtration to virus removal filtration with a filter having a pore size of 10 to 40 nm, and recovering the filtrate].

Claim 17. Amended. A method of removing viral and/or [illegible] proteins from a

pharmaceutical composition of a purified biologically active interferon protein comprising:

(4) adding to a solution of the purified interferon protein a non-ionic detergent;

(5) subjecting the solution containing the non-ionic detergent to filtration on a virus removal filter with a pore size of 10 to 40 nm; and

(6) recovering the filtrate.

Appendix 1. Clarification of specific properties of virus filtration

Virus filtration represents a specific application of membrane filtration and the properties of the membranes used differ greatly from the membranes that are used in ultrafiltration. The virus filter described in the patent application 4,808,315 and which is also used in the examples of the present application consists of a hollow-fibre microporous membrane (Planova®, Asahi Kasei Corporation) constructed of cuprammonium regenerated cellulose. The Planova® membrane is a tortuous, three-dimensional structure of interconnected "voids" and "capillaries" that cannot be easily described with a single index. As indicated by the manufacturer of Planova® filters, protein permeability and virus removability of virus filters can only roughly be estimated using the mean pore size and the only reliable means of determining filter performance for any given application is through a qualification or validation study that simulates actual process conditions. As concerns permeability of detergent solutions, their behaviour is even more unpredictable, because detergents as amphiphilic molecules form micelles, which can be of variable size (e.g. Midura RJ, Yanagishita M. Chaotropic solvents increase the critical micellar concentrations of detergents. Anal Biochem 228(2), 318-322, 1995). The present application is based on the unexpected discovery that a virus removal filter is permeable to polysorbate and similar detergents, and that polysorbate enhances the recovery of sticky biologically active proteins, such as interferons, in the filtrate while maintaining effective removal of small viruses (effective virus removal demonstrated in Table 1).

Table 1. Reduction of B19 DNA in Planova 15N filtration of a solution containing 0.2 g/l polysorbate 20. Geq, genome equivalents; RF, reduction factor.

	Volume ml	B19 DNA geq/ml	B19 DNA total geq	B19 DNA total geq $\log_{10}$	B19 DNA RF $\log_{10}$
B19 spiked solution	44.5	1.0E+06 - 1.0E+07	4.5E+07 - 4.5E+08	7.6 - 8.6	
0.1 µm filtrate	44.1	1.0E+06 - 1.0E+07	4.4E+07 - 4.4E+08	7.6 - 8.6	0 - 0
0.1 µm filtrate	42.6	1.0E+06 - 1.0E+07	4.3E+07 - 4.3E+08	7.6 - 8.6	
Planova filtrate fr. 1	10.2	1.0E+02 - 1.0E+03	1.0E+03 - 1.0E+04		
Planova filtrate fr. 2	10.9	1.0E+02 - 1.0E+03	1.1E+03 - 1.1E+04		
Planova filtrate fr. 3	10.3	1.0E+02 - 1.0E+03	1.0E+03 - 1.0E+04		
Planova filtrate fr. 4	11.3	1.0E+02 - 1.0E+03	1.1E+03 - 1.1E+04		
Planova filtrate	42.7	pool	4.3E+03 - 4.3E+04	3.6 4.6	4.0 - 5.0

The influence of polysorbate on the removal of viruses during virus filtration was studied by using the human parvovirus B19 a model virus. Parvovirus B19 is one of the smallest known viruses (18-20 nm) and it is exceptionally resistant to various physico-chemical inactivation methods, including heat and detergent treatments. Parvovirus is still a common contaminant of blood-derived protein pharmaceuticals and therefore it is very important to develop methods which are effective in removing/inactivating parvovirus B19 in biological products (Schmidt et al. Parvovirus B19 DNA in plasma pools and plasma derivatives, Vox Sang 81, 228-235, 2001). Small physico-chemically resistant viruses may also contaminate animal cell cultures, transgenic animals and animal derived raw materials used in the manufacture of recombinant proteins.

Human serum containing a high amount of parvovirus B19 (about 10^{10} geq/ml) was prediluted 1/1000 with 11 mmol/l sodium phosphate buffer, 140 mmol/l NaCl, pH 7.0 (PBS) and the diluted virus-rich serum was added to 46 ml of PBS containing either 0.2 g/l polysorbate 20. 44.5 ml of the solution was filtered through a 0.1 µm prefilter and a Planova 15N filter (10 cm^2) by using a constant pressure of 0.4 bar at 25°C. The amount of parvovirus B19 DNA in the starting solution, after the prefilter and after the Planova 15N filter was determined by PCR (Söderlund M et al, Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. Lancet, 349, 1063-1065, 1997). The clearance of the virus in the virus filtration step was calculated and expressed as a reduction factor (EU guideline on virus validation studies, CPMP/BWP/268/95).

The reduction factor for parvovirus B19 in the virus filtration step was 4-5 $\log_{10}$ in the PBS solution containing 0.2 g/l polysorbate 20. As the criterium for an effective step in virus removal is considered to be a reduction factor of at least 4 $\log_{10}$ (EU guideline on virus validation studies, CPMP/BWP/268/95), it was concluded that even the very small parvovirus B19 can be effectively removed by virus filtration in the presence of polysorbate 0.2 g/l according to the present application.